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## MEETING REPORT

# ASBMR: cancer and bone

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Meeting Report from The 2014 American Society for Bone and Mineral Research, George R. Brown Convention Center in Houston, TX, USA, 12–15 September 2014.

This year's meeting spanned many bone-related subjects from osteoblasts, osteoclasts, osteocytes, mechanobiology and cancer biology. There were also special focus sections from NASA explaining how to get involved in NASA research, which was a well-attended addition for the Houston meeting. In addition, there were many networking opportunities for members that help make the ASBMR meetings one of the major meetings for interacting with colleagues in the bone field. This meeting report will focus on the Cancer and Bone session, which primarily focused on the Greg Mundy Memorial Session: Cancer and Bone. Overall, the research presented in this and other sessions and the poster sessions was of high quality and very interesting. Within this review I will cover a few of the standout abstracts and sessions that I saw at the meeting, but there were many more excellent abstracts throughout the meeting.

### Introduction

This year the oral sessions returned to previous formats where specific topic areas were together (unlike 2013). I found this year's format easier to find and attend all of the talks that I was interested in seeing. For Cancer and Bone, the primary session was the Greg Mundy Memorial Session: Cancer and Bone on Saturday 14th September, chaired by Drs Roodman (Indiana University) and Sterling (Vanderbilt University). This session contained six talks selected from abstract submissions. The American Society for Bone and Mineral Research (ASBMR) was a meeting that Dr Mundy looked forward to, and I am certain that he would be honored to have this session in his memory. The session was well-attended and the talks were of high quality, with a good amount of audience participation. In addition to this session, there were a few other cancer talks scattered throughout the meeting. Although the talks were all excellent, there was a distinct lack of myeloma talks in the primary session. This year prostate cancer seemed to be a major focus with three out of the six talks in the Cancer and Bone Session focusing on prostate cancer (two breast and one osteosarcoma talks filling out the remaining three talks). As always, there were many posters covering a variety of topic areas and tumor types. The topic areas of this year's meeting were fairly broad and mostly centered around muscle and bone interactions, signaling in tumor-induced bone disease, the tumor microenvironment, potential therapeutics, and mechanical stimulation and bone (poster session).

### Muscle and Bone

Dr Theresa Guise presented a fascinating talk (Friday at 11:30: Symposium—Muscle and Bone) on how tumors in bone affect muscle and cachexia. Although she and her colleagues have presented some of this work at previous meetings, it was great to see it all together, and they seemed to have new data since last year. She showed that the muscle from mice with tumors in bone has leaky calcium channels (RyR1, Ryanodine receptor1) caused by oxidative stress in the microenvironment. This causes an overall muscle weakness in the animals even at sites distant from the tumor-bearing bone. She compared this phenomenon with muscular dystrophy, and used the drug Rycal to prevent the leaky calcium channels, which successfully blocked the muscle weakness associated with tumor burden in bone. Their work also showed that transforming growth factor-β (TGF-β) signaling (through an increase in pSmad3) in the muscle was increased in tumor-bearing mice. This led to testing SD-208 (a TGF-β receptor type I kinase inhibitor) and zoledronic acid in tumor-bearing mice. Both blocked the muscle weakness observed in tumor-bearing mice and blocked the RyR1 and the calcium leak. Finally, their work showed that Nox4 was the mediator of the TGF-β-induced calcium leakage, and shRNA for Nox4 blocked the calcium leak. This talk stimulated an active group of questions that were very interesting. Steve Harris from San Antonio asked whether they had looked into differences in food intake between tumor- and non-tumor-bearing mice and accounted for this in their studies. She discussed that they had calorie restricted some mice, which reduced muscle bulk, but it did not affect strength to the extent seen in tumor-bearing mice. These studies point to a potential therapeutic strategy for reducing muscle weakness in cancer patients.

### Tumor Signaling

Many of the oral presentations and posters focused on basic signaling mechanisms behind tumor metastasis and growth in

bone. These types of studies have historically led to potentially new therapeutic strategies for treating tumor-induced bone disease and are important for continuing to improve our understanding bone disease and for developing cutting-edge, novel therapeutic approaches. In the first oral presentation in the Greg Mundy Memorial session (1033), Edith Bonnelye described her work on estrogen-related receptor α (ERRα) expression in prostate cancer metastasis to bone. Her work demonstrated a strong correlation between the expression of ERRα expression and bone metastases in prostate cancer patients. To confirm this, they modulated its expression in PC-3 cells and found that overexpression increased bone destruction, and surprisingly they found that these cells caused a mixed lesion with bone destruction and bone formation, and their data suggested an alteration in the interaction of these cells with the bone microenvironment. This suggests an important role for ERRα in prostate cancer cells and makes an interesting model for studying mixed prostate cancer lesions.

Another abstract in this session presented by Stefanie Thiele (1034) focused on WNT5A expression in prostate cancer metastasis to bone. They demonstrated that WNT5A expression was higher in patients with prostate cancer compared with patients with benign disease. However, patients with higher expression of WNT5A showed an increase in survival versus those with lower levels of expression. Supporting the clinical findings, they showed that overexpression of WNT5A in prostate cancer cells reduced tumor cell proliferation and reduced bone metastasis in the PC-3 cells. This talk stimulated many questions regarding how WNT5A overexpression by the tumor cell altered the bone microenvironment and how it might alter bone formation. Dr Thiele responded that they plan to look at these effects in future experiments.

The abstract presented by Chunxi Ge (1036) focused on Runx2 in prostate cancer invasion. He specifically examined the importance of the Ser 301 and Ser 319 phosphorylation sites in the downstream production of metastasis-associated genes, which he showed were important to stimulate *in vitro* invasion and metastasis of prostate cancer cells. In patient samples they demonstrated that phosphor-specific staining correlated with the aggressiveness of prostate tumors.

In addition, many of the posters focused on signaling pathways. Popular pathways included Wnt, Hedgehog (HH), Runx2 and many others. One standout signaling poster was presented by Dr Gregory Clines (SA0079). His poster focused on CXCL14 and showed that it was upregulated in prostate cancer bone metastases to compared to soft tissue metastases and may be important for tumor colonization of the bone.

## Tumor Microenvironment

As abstracts and presentations addressing the tumor microenvironment have dominated ASBMR in the past several years, there were surprisingly few abstracts that focused specifically on the tumor microenvironment. Unlike last year where the entire Greg Mundy Memorial session centered on the microenvironment, this year there was more of a focus on signaling pathways. Of the microenvironment posters, Dr Laurie McCauley's (SA0072-Listed as Jacqueline Jones as the presenter, who unfortunately could not attend) contained the most novel hypothesis. In this study, they investigated how macrophages regulate skeletal metastasis. They specifically focused on the role of macrophages at cleaning apoptotic debris (efferocytosis) and demonstrated that the efferocytic function of the M2 macrophages supported prostate cancer growth, suggesting that inhibiting these subpopulations of cells may be a therapeutic approach for inhibiting tumor-induced bone disease.

While in previous years many posters focused on the myeloid-derived suppressor cells (MDSCs) in bone metastatic disease, this year there were only three abstracts focusing on MDSCs. One of these abstracts presented by Lucia D'Amico (SA0077) investigated the downregulation of β-catenin observed in MDSCs from patients and mice, which enhances their ability to promote tumor growth. They demonstrated that DKK1 was responsible for this reduction, and that inhibiting DKK1 reduced tumor growth and the % of MDSCs.

Another group focused on the role of osteoblasts in the development of tumor-induced bone disease. In this abstract by Aruna Kode (1088), they investigated the role of FoxO1 deletion (one allele) from the osteoblasts on the development of acute myeloid leukemia. Using this approach they observed a reduction in anemia, neutrophilia and lymphocytopenia that was observed in the wild-type mice. In addition, this deletion extended the survival of the mice, and no signs of disease were observed during a 1-year observation period.

Finally, one presentation by Paul Daft (1087), investigated the role of exosomes in the regulation of recurrence in osteosarcomas. They found that a small population of the tumor, the tumor initiating cells, led to recurrence and that these cells produced α-CaMK-II and exosomes. Adding these exosomes to the dormant tumor cells significantly increased Notch 1 and stimulated the cells out of dormancy, suggesting that these exosomes may be an important factor for recurrence in osteosarcoma.

## Tumor Models

Despite decades of studies and many very informative models, there are still many improvements that can be made to some of the current models to allow for more complex studies of tumor signaling and interactions with the bone microenvironment. One tumor type where improvements in current models would greatly impact our ability to study tumor-induced bone disease is osteosarcomas. In Saturday's Cancer and Bone session Jianning Tao (1035) presented his studies on developing a spontaneous osteosarcoma model. They developed this model using a conditional mouse model of truncated Notch1. Using this model, they found that all mice developed bone tumors, which were accelerated by p53 loss. This is a promising model for studying the development of osteosarcoma and could help explore mechanisms and potential therapeutics.

Dr Capietto presented an interesting poster (SU0070) where she described a model of estrogen receptor-α positive (ER+) mammary tumor. This is especially important as many patients with bone metastases have ER+ tumors; yet, all of the current cell lines and mouse models rely on ER-tumor cells. She generated two cell lines from STAT1$^{-/-}$ mouse (SSM2 and SSM3) that could metastasize to bone. One model remained ER sensitive *in vivo*, whereas the other became ER insensitive

(but still ER +) when residing in bone. This is an appealing model for studying the role of ER in metastatic bone disease.

With the current emphasis on patient-derived xenografts (PDXs) there is significant interest in developing a model where bone metastatic patient cells could be grown in mice. One group set-out to develop such a model. In a poster by Eliza Fong (SU0068) a group at Rice University demonstrated that they could grow bone metastatic prostate cancer cells in a hyaluranan (HA) hydrogel scaffold subcutaneously in severe combined immunodeficiency mice. These scaffolds were intended to mimic the bone marrow microenvironment rather than the bone. The group also investigated the growth of human bone marrow stromal cells and murine pre-osteoblasts in their three-dimensional (3-D) model and showed that they could support tumor growth. This is a potentially interesting system for developing a PDX model for bone metastases, and it will be interesting to see whether this model supports more complex primary human tumors.

Another 3-D model was presented by Ushashi Dadwal (MO166). Unlike the HA hydrogels, Ms. Dadwal's 3-D scaffolds (polyurethanes) replicated the pore size, flow rate and rigidity of the bone (opposed to the marrow) and tested the growth of bone metastatic tumor cells. Her work demonstrated that bone metastatic tumor cells move faster on rigid scaffolds and that expression of genes associated with bone destruction was upregulated in the more rigid 3-D scaffolds opposed to the softer scaffolds. This 3-D model can be used in a perfusion bioreactor or *in vivo* to measure changes in gene or protein expression. This model has the potential to allow researchers to investigate cell–cell interactions in the context of a bone-mimicking microenvironment and suggests that rigidity is a key factor in regulating the gene expression of bone metastatic cells.

## Potential Therapeutics

New therapeutic approaches are always a popular topic during the oral session, and this year's Cancer and Bone Session had several talks that focused on both novel therapeutics and better understanding current therapies. Dr Taipaleenmaki presented her work targeting Runx2 in metastatic breast cancer using micro-RNA (miRNA) (1037). They identified two miRNAs, miR-135 and miR-203, as inhibitors of Runx2 expression. Overexpression of these miRNAs in the tumor cells led to a decrease in tumor growth, bone metastasis and bone destruction, suggesting that delivery of miRNAs may be a potential therapeutic approach for inhibiting tumor-induced bone disease.

The final talk in the Cancer and Bone session was presented by Dr Michael Rogers (1038) and focused on examining how bisphosphonates (BPs) may reduce tumor growth and metastasis outside of the skeleton. To do this he used intravital 2-photon imaging to image mammary tumors treated with fluorescently labeled bisphosphonates. In my opinion, his talk was one of the best of the meeting. He showed numerous movies that convincingly demonstrated the binding of bisphosphonates to calcified nodules within the mammary tumor, which was mediated by leaky vasculature near the tumor. These calcium bound bisphosphonates were then engulfed by the tumor-associated macrophages (TAMs), suggesting that the anti-tumor effect of BPs may be due to the alteration of interactions between tumor cells and the TAMs rather than directly on the tumors. This work is currently in press in Cancer Discover.[1]

Dr Rebecca Silbermann presented interesting work in an oral poster presentation (FR-0071) that tested a small molecule inhibitor (XRK3F2) that blocks bone marrow stromal interactions (BMSC) with multiple-myeloma (MM) cells by inhibiting the p62 ZZ domain-mediated protein interactions. When XRK3F2 was used in co-cultures of MM and BMSCs, the expression of Runx2 increased in the BMSCs and induced new bone formation in MM animal models, suggesting that this drug may be able to stimulate new bone formation in MM bearing bones.

Several posters also focused on therapeutic strategies. One poster (MO0073) explored whether chronic treatment with Abaloparatide (a synthetic hPTHrP analog) would have a similar effect on increasing osteosarcomas in rats (increase incidence not seen in humans) as prolonged treatment with parathyroid hormone (PTH) (1–34), and whether this could be a potential osteoporosis treatment for patients with risk factors that would prevent them from using PTH. Unfortunately, they saw a similar increase in osteosarcomas in the rats with Abaloparatide. However, it is unclear whether a similar effect would be seen clinically.

Finally, Dr Philippe Clezardin's group presented a poster (SA0084) describing Integrin $\alpha5\beta1$ as a target to treat breast cancer metastasis to bone. The $\alpha5\beta1$ antibody, M200, delayed the onset and extent of tumor burden and bone destruction and reduced osteoclast differentiation. This suggests $\alpha5\beta1$ inhibition as a potential therapeutic approach for inhibiting tumor growth and bone destruction.

## Mechanical Stimulation and Effects on Cancer

This year one of the major themes throughout the meeting was mechanobiology. Although most of these talks were in chondrocytes, osteocytes or other bone cells, there were a few posters in the cancer and bone posters that explored mechanical stimulation and the effects on cancer-induced bone disease.

A poster presented by Dr Gabriel Pagnotti (MO0083) explored how low-intensity vibration (LIV) can protect the skeleton in MM animal models. He showed that the LIV-treated mice displayed a reduction in infiltrating disease and increased bone volume/total volume (BV/TV), suggesting that LIV may protect MM tumor-bearing bones.

Ms Shellese Cannonier (MO0078) presented a poster on signaling pathways that regulate oral squamous cell carcinoma. This work suggested that the rigidity of the mandible alters the expression of genes associated (Gli2 and PTHrP) with oral cancer invasion, suggesting that mechanical stimulation may alter genes that can enhance bone destruction. Similarly, another group (MO0069) investigated that Sonic Hedgehog (Shh) was highly expressed in stromal cells and osteoblasts near oral tumors that had invaded the bone, and that Gli2 was expressed in the osteoclasts, which led to an increase in bone destruction, suggesting that oral cancer alters the bone microenvironment.

As osteocytes are well-established to be sensitive to mechanical signaling, one group looked at the interactions of mechanically loaded osteocyte-like cells (MLO-Y4) and MDA-MB-231 breast cancer cells. These studies demonstrated that MDA-MB-231 cells migrated more toward the conditioned media from mechanically stimulated osteocytes than control

osteocytes, and displayed a reduction in apoptosis. Similar to Ms. Cannonier's poster, this suggests that increased loading may not prevent bone metastases and that the effect of mechanical stimulation on tumor cells is likely to be complex.

Although it did not directly investigate mechanical signaling, another interesting poster (SU0071) investigated the role of insulin-like growth factor 1 (IGF-1) and focal adhesion kinases (FAKs) on angiogenesis in bone metastatic disease. FAKs are a key component to mechanical signaling, and this abstract demonstrated that a dual inhibitor (FAK and IGF-1, TAE226) reduced bone metastasis and the accumulation of CD31+ endothelial cells within the tumor.

## Other Interesting Abstracts

Another area that has been popular in previous meetings, but had few abstracts this year was cancer and pain. Sam Olechnowicz (SU0083) presented a poster on MM-related pain. In this abstract, the authors used an activator of adiponectin (a molecule that the authors previously published to be protective in MM), L-4F. This drug reduced tumor burden but also reduced the expression of the pain mediator nerve growth factor (NGF) in osteoblasts and stromal cells, suggesting that it could be a potential therapeutic strategy to reduce tumor burden and pain.

Although not directly cancer related, I found the International ONJ task force (Sunday 1200–1230 hours) session to be very informative. This session was co-chaired by Drs Laurie McCauley (University of Michigan) and Tetradis (University of California, Los Angeles) and had three speakers. Dr Compston discussed Diagnosis and Pathophysiology, Dr Khan discussed Incidence and Prevention Strategies, and Dr Morrison discussed Staging and advances in management. They concluded that, although there remain varying reports on incidence rates of ONJ that cancer patients on zoledronic acid or denosumab have the highest incidence rate (1–2%), but that anti-angiogenesis drugs (such as avastin) can induce similar effects. Importantly, they strongly emphasized that the risk of developing ONJ is small in comparison with the large benefit in the reduction in skeletal-related events in these patients. They also emphasized that the outcomes for these patients have been drastically improved over the past decades by paying more attention to oral hygiene in these cancer patients.

## Conclusion

Although the 2014 meeting was an excellent meeting, there seemed to be fewer cancer orals this year than in previous years. There also seems to be a greater focus on basic mechanisms of signaling pathways, which may explain the reduction in oral presentations. However, based on the high quality of many of the posters, I think we can look forward to some strong oral presentations developed from these basic studies over the next few years. I look forward to seeing the progression of these data next year in Seattle!

## Conflict of Interest

The author declares no conflict of interest.

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## Reference

1. Junankar S, Shay G, Jurczyluk J, Ali N, Down J, Pocock N *et al.* Real-time intravital imaging establishes tumour-associated macrophages as the extraskeletal target of bisphosphonate action in cancer. *Cancer Discov* 2014; **5**: 35–42.